that peptides in the I-state form pores, while peptides that remain in the S-state use the carpet model of membrane disruption.
Here, OCD is used to investigate the orientation and threshold concentration of Piscidin 1 and Piscidin 3 (P3) in bacterial and mammalian-mimicking lipid systems and thereby determine the mechanism of action of P1 and P3 in these lipid systems. Both peptides are a 22-residue alpha-helical AMPs isolated from the mast cells of hybrid striped sea bass. P1 is both more antimicrobial and hemolytic than P3. We hypothesize that the two peptides behave differently in bacterial versus human cells due to the differences in membrane composition and that P1 initiates its activity at a lower threshold concentration. Mammalian and bacterial membrane mimics have been made using 4:1 PC/CHL (phosphocholine and cholesterol, respectively) and 3:1 PC/PG (phosphoglycerol). The bilayer orientations of piscidin have been investigated over a large range of P/L ratios using OCD. Membrane thinning was studied by x-ray. These studies provide insight into the mechanism of action of an important class of AMPs and may help provide design principles for new drug candidates.

1236-Pos Board B128
Surface and Membrane Binding Properties of the Lipopeptide Daptomycin
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Daptomycin, an antimicrobial lipopeptide used to treat infections caused by Gram-positive bacteria that are resistant to many conventional therapies, acts through calcium-mediated binding to and rapid depolarization of the target bacterial membrane. Convincing evidence has recently been reported suggesting that small daptomycin oligomers form at the membrane surface and that these complexes represent the active state of the drug. Daptomycin's activity is closely correlated with the presence of phosphatidylglycerol (PG) in the target membrane. Although there have no cases of clinical resistance to daptomycin reported, troubling signs are emerging indicating that changes in lipid composition of bacterial membranes cause decreased susceptibility to the drug. It is therefore of interest to gain a more profound understanding of the details of daptomycin's mechanism of activity at the membrane level and the possible causes of potential resistance and their relationship to lipid composition. In the current study, we report on our investigation into the surface and membrane binding properties of daptomycin. From the Gibb's adsorption isotherm, we estimate the molecular area of daptomycin at the air-aqueous interface. Using Langmuir monolayers as membrane models, we also report limiting surface pressures and kinetics for daptomycin insertion to lipid films comprised of pure PG or PG-phosphatidylcholine mixtures. Finally, we attempt to correlate daptomycin's binding behavior in monolayers to that in bilayers, in the form of unilamellar vesicles, by presenting results from isothermal titration experiments. The results represent, for the first time, thermodynamic binding parameters for daptomycin-membrane interactions.

1237-Pos Board B129
Effects of Electrostatic Interactions on Helicity in Model Peptide Antibiotics using 2D NMR and CD Spectroscopies
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Persistent infections caused by antibiotic resistant microbes are a serious public health threat. Peptide antibiotics, which perturb the cell membrane, offer one promising solution. Critical characteristics of both natural and designed peptide antibiotics include the formation of ordered structures such as helices, and amphilicity. We are investigating the effects of electrostatics on the helicity of peptide antibiotic models that are composed primarily of the sterically hindered amino acid Aib, with Lys and Glu residues substituted at various positions in the helix. We report here results for the octameric peptides KK36 (Lys at positions 3 and 6, one 310-helical turn apart), EK36 (Glu and Lys at positions 3 and 6), EK45 (Glu and Lys adjacent in the center of the helix) and KK45 (two Lys adjacent in the center of the helix). NMR resonances were assigned using natural abundance 1H-13C HMBC and HSQC spectra. Distance constraints from 1H-1H ROESY and hydrogen-bonding information from amide temperature coefficients were used to calculate the three-dimensional structures of the peptides using Xplor-NIH. Global structural information was also obtained using CD spectroscopy. We find that KK36 and EK36 are 310-helical, with slightly different helical curvatures both in DMSO-d6 and in methanol-d3. CD spectra also indicate 310-helical structures in TFE and methanol, with a greater ratio of θ220/θ202 in TFE (~0.4 versus ~0.25). KK36 is less well structured in water versus organic solution, as evidenced by poor amide spectral dispersion. However, association of KK36 with lipid vesicles results in complete amide spectral dispersion, and an enhancement of the CD signal. The structures of all four peptides will be presented and interpreted in terms electrostatic effects on helicity in a variety of solvent systems.

1238-Pos Board B130
Channel Crystal Structure and Antimicrobial Mechanism of Dermcidin from Human Skin
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Dermcidin is an anionic antimicrobial peptide (aAMP) derived from the human gene DCD, which encodes a preform that is secreted by eccrine sweat glands and subsequently proteolytically processed into DCD-1L, constituting a vital part of the innate host defense of the dermis. Recently its oligomeric structure was solved by x-ray crystallography and shown to be stabilized by divalent Zn^{2+} ions. Through molecular dynamics simulations, using a novel transmembrane conduction assay, we showed that it forms a conducting channel in a lipid bilayer. Long standing experimental results assign it a selective affinity for negatively charged membranes despite its anionic nature (pI ~5), and in consensus with other amphiphilic AMPs it has a helical structure that only forms upon contact with a hydrophobic interface. The reconciliation of such experimental observations with the mechanistic result of channel formation pose further questions on the selectivity of dermcidin, both for anion conductivity and bacterial membrane adhesion, and for the kinetics of its insertion. Working to understand how the subunits of this channel-forming oligomer interact, we use molecular dynamics simulations to examine the influence and specific role of the Zn^{2+} ions for its stability under conditions relevant to understand it's selectivity for (and insertion into) specific composition lipid bilayers. In extension we examine the effect specific residue mutations which are based on a structure- and sequence-comparison to other known AMPs has for its function, as well as that of lipid bilayer composition.

Membrane Structure I

1239-Pos Board B131
Influence of Divalent Cations on Phosphatidylserine Lipid Flip-Flop
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Phosphatidylserine (PS) lipids are an essential component of the plasma membrane of eukaryotic cells and are known to distribute unequally across the membrane. Changes to this distribution trigger specific cell functions, ranging from blood coagulation to phagocyte recognition, while defects in the distribution are linked to disease. Despite many research efforts, the mechanism to control the PS lipid distribution has proven to be complex and is not yet fully understood. While there are many biological interactions which may contribute to PS distribution, the work presented here investigates the role of divalent cations. Sum-frequency vibrational spectroscopy (SFVS) has been used to study the changes in native lipid behavior induced by the presence of both magnesium and calcium ions. Planar-supported lipid bilayers containing biologically relevant amounts of PS lipids (5-20%) were used as model systems to isolate the impact of these divalent cations on lipid flip-flop and PS distribution.

1240-Pos Board B132
The Thermodynamics of General and Local Anesthesia
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We describe the influence of both local and general anesthetics on the melting transition in lipid membranes. We outline the theory of the interaction and compare it to calorimetric experiments. We found that both local and general anesthetics display very similar effects on membrane melting that can be described by the well-known phenomenon of melting point depression. The partitions coefficients of the anesthetics can be deduced from the calorimetric profiles. We also investigate the influence of hydrostatic pressure on the anesthetic effect. Both classes of anesthetics display pressure reveal, an effect that has been found for general anesthesia but has not been described for local anesthetics. Our findings are in agreement with recent clinical findings by Danish neuroscientists from the Imperial Hospital in Copenhagen (Rigshospitalet) on the excitability of the human median nerve that were performed in collaboration with